SHORT REPORT

Influence of GSTT1 null genotype on the offspring sex ratio of gasoline filling station workers

M Ansari-Lari, M Saadat, N Hadi

J Epidemiol Community Health 2004;**58**:393–394. doi: 10.1136/jech.2003.011643

The aim of this study was to investigate whether the occupational exposure to gasoline of men employed at filling stations affects the sex of their children. Altogether 115 offspring (47 males, 68 females) were identified within families of 49 men working in filling stations in Shiraz (Fars province, south of Iran) and 345 offspring (178 males, 167 females) from 147 families of unexposed persons from the general population of Shiraz, which were matched by age of fathers (±2 years) and number of children as a control group. The offspring sex ratio at birth (male proportion) in the filling station workers was significantly lower than the ratio in control group (OR = 0.65; 95% confidence intervals (CI) 0.42 to 0.99). Genotypes of glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) were investigated on extracted genomic DNA of 37 exposed workers using the polymerase chain reaction based method. In exposed group with active GSTM1 and GSTT1 genes, offspring sex ratio was the same as the ratio in the control group (OR = 0.66; 95% CI 0.34 to 1.28). However, in the exposed group with active GSTM1 and null genotype of GSTT1, the offspring sex ratio statistically decreased (OR = 0.45; 95% CI = 0.21 to 0.96). It seems that the GSTT1 null genotype has an effect on offspring sex ratio in the filling station workers.

Many demographic and environmental factors have been shown to be associated with variation in human sex ratio at birth. It has been shown that exposure to environmental toxins changes the sex ratio of live births in humans.[1] There are several studies that have shown the association between fathers' occupation and sex ratio of offspring.[2–4] Male reproductive hazards may usefully be monitored by low offspring male to female ratio.[2]

The glutathione S-transferases (GSTs) are involved in cellular detoxification.[5 6] The GSTs have been divided into a number of subclasses, α, μ, π, and θ. There are well defined genetic polymorphisms in the expression of GSTM1 (a member of class μ) and GSTT1 (a member of class θ) enzymes with non-functional null-alleles named GSTM1-0 and GSTT1-0, respectively. The homozygosity for these null-alleles is associated with the absence of the corresponding enzyme activity.[5 6] The GSTs involved in detoxification of several toxins including some of compounds present in gasoline.[7] On the other hand, the reported data provide some evidence of an association between GSTs polymorphisms and risk of recurrent early pregnancy loss.[8 9] Therefore, we speculate that paternal GSTM1 and GSTT1 genotypes might be associated with offspring sex ratio in gasoline filling station workers, who have long term exposure to gasoline.

METHODS

Using a simple questionnaire, number of sons and daughters of 49 men working in filling stations in Shiraz (Fars province, south of Iran) were determined. We identified 115 offspring (47 males, 68 females) within these families. The mean duration of employment in the filling station was 7.7 years (range 1.8–29 years). Because it is reported that paternal age and birth order have some effect on offspring sex ratio,[3 10] for each exposed subjects, three unexposed persons from the general population of Shiraz (without occupational exposure to gasoline) were matched by age of fathers (±2 years) and number of children as a control group. In the control group, 345 offspring (178 males, 167 females) were identified.

Blood samples were obtained from 37 of 49 filling station workers participating in the study. Genomic DNA was isolated from whole blood. The polymerase chain reaction (PCR) conditions for determining GSTM1 and GSTT1 genotypes were the same as that reported previously.[5 6] The absence of amplified product was consistent with the null genotype. Successful amplification by β-globin specific primers confirmed the proper function of the PCR reaction.[11]

The sex ratio expressed as the proportion of total live births that were male (male proportion). The odds ratio (OR) (the odds of being male) and 95% confidence intervals (CI) were calculated. An OR>1.0 indicates an increase in the sex ratio and an OR<1.0 indicates a decrease in the sex ratio. Comparison of paternal age and number of children was done using two tailed unpaired Student's *t* test. A probability of $p<0.05$ was considered a significant difference.

RESULTS

The sex ratio at birth of exposed and unexposed groups were 0.409 (total birth = 115) and 0.516 (total birth = 345), respectively. The offspring sex ratio at birth in the filling station workers was significantly lower than the ratio in the control group (OR = 0.65; 95% CI = 0.42 to 0.99).

Table 1 shows the association of paternal GSTM1 and GSTT1 genotype combinations and offspring sex ratio in exposed group. Because it is reported that in general population, the GSTM1 and GSTT1 genotypes and their combinations showed no association with offspring sex ratio,[11] the sex ratio in each combination, was compared with the control group. The offspring sex ratio in exposed group with active GSTM1 and GSTT1 genes was the same as the ratio in control group (OR = 0.66; 95% CI = 0.34 to 1.28). However, when the GSTT1 became inactive (active GSTM1 and null genotype of GSTT1) the sex ratio in the offspring significantly decreased (OR = 0.45; 95% CI = 0.21 to 0.96). Statistical analysis for the two other combinations was not performed, because the sample size was too small.

It should be noted that there was no statistical difference for paternal age ($t = 0.86$, df = 28, $p = 0.40$) and number of children ($t = 0.72$, df = 28, $p = 0.48$) between the group with two active genes and the group with active GSTM1 and GSTT1 null genotype.

Abbreviations: PCR, polymerase chain reaction; GST, glutathione S-transferase

Table 1 Association between combination of paternal GST genotypes and offspring sex ratio in exposed group

Combination of genotypes		Sex of offspring			
GSTM1	GSTT1	Sons	Daughters	OR	95% CI
Present	Present	17	24	0.66	0.34 to 1.28
Present	Null	11	23	0.45	0.21 to 0.96
Null	Present	3	4	NT*	–
Null	Null	3	3	NT	–

The control group assumed as reference (OR = 1.0). *Because of the small sample size OR were not determined.

DISCUSSION

It is reported by Yang *et al* that the association between exposure to petroleum air pollution near a petroleum refinery plant in Taiwan and sex ratio at birth was not significant.[12] Recently we reported the increased sex ratio of live births in Masjid-i-Sulaiman, south west of Iran, which is contaminated by sub-surface leakage of natural gas containing hydrogen sulphide.[13] The present results showed that sex ratio decreased in the offspring of filling station workers. The increased sex ratio in Masjid-i-Sulaiman, however, can be attributed to the exposure of both parents to the toxin.

Recently, we reported that in the general population, the GSTM1 and GSTT1 genotypes and their combinations showed no association with offspring sex ratio.[11] Our results may indicate that the offspring sex ratio is associated with combination genotypes of GSTM1 and GSTT1 of exposed group. It is reported that GSTT1 is involved in detoxification of several toxins including some of the compounds present in gasoline.[7] A possible explanation for the change in sex ratio when the fathers have null genotype of GSTT1/active GSTM1 is disruption of detoxification processes of some gasoline toxins or their metabolites. In this status conditions became unfavourable. The finding reported here is in agreement with the hypothesis of Trivers and Willard. They hypothesised that under favourable environmental conditions males would be favoured and that under adverse conditions females would be favoured in polygynous species.[14] There is some evidence that supports the Trivers and Willard hypothesis in human populations.[15 16] A few strong men could sire many grandchildren, whereas women have a more sure but limited reproductive potential. Taken together, it is suggested that under unfavourable conditions, the null genotype of GSTT1 shows a selective advantage.

Authors' affiliations
M Ansari-Lari, N Hadi, Department of Community Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
M Saadat, Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran

Funding: this study was supported by Shiraz University.
Conflicts of interest: none declared.

Correspondence to: Dr M Saadat, Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran; saadat@susc.ac.ir

Accepted for publication 28 July 2003

REFERENCES

1 **James WH**. The human sex ratio. Part 1: a review of the literature. *Hum Biol* 1987;**59**:721–5.
2 **Davis DL**, Gottlieb MB, Stampnitzky JR. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? *JAMA* 1998;**279**:1018–23.
3 **James WH**. Offspring sex ratio as a potential monitor of reproductive disorders in communities near hazardous chemical sites. *Reprod Toxicol* 1997;**11**:893–5.
4 **Milham S Jr**. Unusual sex ratio of birth to carbon setter fathers. *Am J Ind Med* 1993;**23**:829–31.
5 **Pemble S**, Schroeder KR, Spencer SR, *et al*. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994;**300**:271–6.
6 **Harada S**, Misawa S, Nakamura T, *et al*. Detection of GST1 gene deletion by the polymerase chain reaction and its possible correlation with stomach cancer in Japanese. *Hum Genet* 1992;**90**:62–4.
7 **Sorsa M**, Osterman-Gokar S, Peltonen K, *et al*. Assessment of exposure to butadiene in the process industry. *Toxicology* 1996;**113**:77–83.
8 **Zusterzeel PL**, Nelen WL, Roelofs HM, *et al*. Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. *Mol Hum Reprod* 2000;**6**:474–8.
9 **Hirvonen A**, Taylor JA, Wilcox A, *et al*. Xenobiotic metabolism genes and the risk of recurrent spontaneous abortion. *Epidemiology* 1996;**7**:206–8.
10 **Erickson JD**. The secondary sex ratio in the United States 1969–71: association with race, parental ages, birth order, paternal education and legitimacy. *Ann Hum Genet* 1976;**40**:205–12.
11 **Saadat M**, Saadat I. Offspring sex ratio is not associated with parental GSTT1 and GSTM1 null genotypes. *Reprod Toxicol* 2003;**17**:345–7.
12 **Yang CY**, Cheng BH, Hsu TY, *et al*. Female lung cancer mortality and sex ratio at birth near a petroleum refinery plant. *Environ Res* 2000;**83**:33–40.
13 **Saadat M**, Ansari-Lari M, Bahaoddini A. Sex ratio at birth in Masjid-i-Sulaiman (Khozestan province, Iran). *Occup Environ Med* 2002;**59**:853.
14 **Trivers RL**, Willard DE. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 1973;**179**:90–2.
15 **Koziel S**, Ulijasaek SJ. Waiting for Trivers and Willard: do the rich really favor sons? *Am J Phys Anthropol* 2001;**115**:71–9.
16 **Ansari-Lari M**, Saadat M. Changing sex ratio in Iran, 1976–2000. *J Epidemiol Community Health* 2002;**56**:622–3.